pRL4

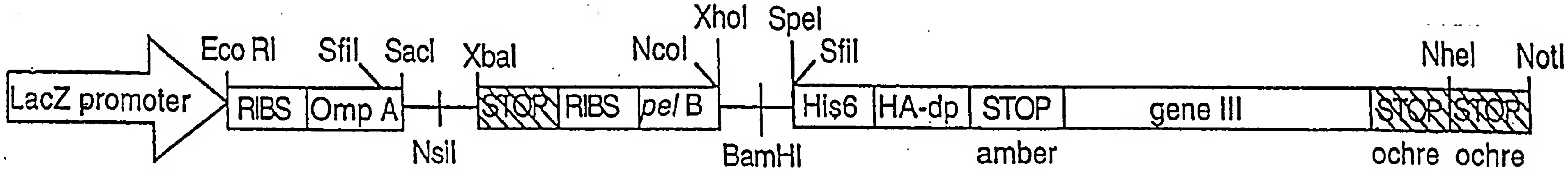
LacZ promoter
Eco RI
RIBS
SfiI
Omp A
SacI
NsiI
XbaI
STOP
RIBS
pel B
NcoI
XhoI
BamHI
SpeI
SfiI
His6
HA-dp
STOP
amber
gene III
NheI
STOP
STOP
NotI
ochre
ochre

Fig. 1

Human Antibody sequence (TT sequence) (SEQ ID NO:54 )

Heavy Chain: cloning sites Xho I and Spe I are underlined

```
1                                            11
gag gtg cag ctg CTC GAG CAG TCT GGG GCT GAG GTG AAG AAG CCT GGG TCC TCG GTG AAG
glu val gln leu leu glu gln ser gly ala glu val lys lys pro gly ser ser val lys
21                                           31
GTC TCC TGC AGG GCT TCT GGA GGC ACC TTC AAC AAT TAT GCC ATC AGC TGG GTG CGA CAG
val ser cys arg ala ser gly gly thr phe asn asn tyr ala ile ser trp val arg gln
41                                           51
GCC CCT GGA CAA GGG CTT GAG TGG ATG GGA GGG ATC TTC CCT TTC CGT AAT ACA GCA AAG
ala pro gly gln gly leu glu trp met gly gly ile phe pro phe arg asn thr ala lys
61                                           71
TAC GCA CAA CAC TTC CAG GGC AGA GTC ACC ATT ACC GCG GAC GAA TCC ACG GGC ACA GCC
tyr ala gln his phe gln gly arg val thr ile thr ala asp glu ser thr gly thr ala
81                                           91
TAC ATG GAG CTG AGC AGC CTG AGA TCT GAG GAC ACG GCC ATA TAT TAT TGT GCG AGA GGG
tyr met glu leu ser ser leu arg ser glu asp thr ala ile tyr tyr cys ala arg gly
101                                          111
GAT ACG ATT TTT GGA GTG ACC ATG GGA TAC TAC GCT ATG GAC GTC TGG GGC CAA GGG ACC
asp thr ile phe gly val thr met gly tyr tyr ala met asp val trp gly gln gly thr
121                                          131
ACG GTC ACC GTC TCC GCA GCC TCC ACC AAG GGC CCA TCG GTC TTC CCC cTG GCA CCC TCC
thr val thr val ser ala ala ser thr lys gly pro ser val phe pro leu ala pro ser
141                                          151
TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC
ser lys ser thr ser gly gly thr ala ala leu gly cys leu val lys asp tyr phe pro
161                                          171
GAA CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG
glu pro val thr val ser trp asn ser gly ala leu thr ser gly val his thr phe pro
181                                          191
GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC
ala val leu gln ser ser gly leu tyr ser leu ser ser val val thr val pro ser ser
201                                          211
AGC TTG GGC ACC CAG ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG
ser leu gly thr gln thr tyr ile cys asn val asn his lys pro ser asn thr lys val
221                                          231
GAC AAG AAA GTT GAG CCC AAA TCT TGT GAC AAA act agt
asp lys lys val glu pro lys ser cys asp lys thr ser
```

10065593.120501

Fig. 2A

Human Antibody Sequence (TT sequence) (SEQ ID NO: 55)

Light Chain: cloning sites Sac I and Xba I are underlined

```
1                                                 11
gag ctc acg cag tct cca ggc acc ctg tct ttg tct ccA ggg gaa aga gcc acc ctc tcc
glu leu thr gln ser pro gly thr leu ser leu ser pro gly glu arg ala thr leu ser
21                                                31
tgc agg gcc agt cac agt gtt agc agg gcc tac tta gcc tgg tac cag cag aaa cct ggc
cys arg ala ser his ser val ser arg ala tyr leu ala trp tyr gln gln lys pro gly
41                                                51
cag gct ccc agg ctc ctc atc tat ggt aca tcc agc agg gcc act ggc atc cca gac agg
gln ala pro arg leu leu ile tyr gly thr ser ser arg ala thr gly ile pro asp arg
61                                                71
ttc agt ggc agt ggg tct ggg aca gac ttc act ctc acc atc agc aga ctg gag cct gaa
phe ser gly ser gly ser gly thr asp phe thr leu thr ile ser arg leu glu pro glu
81                                                91
gat ttt gca gtg tac tac tgt cag cag tat ggt ggc tca ccg tgg ttc ggc caa ggg acC
asp phe ala val tyr tyr cys gln gln tyr gly gly ser pro trp phe gly gln gly thr
101                                               111
AAG GTG GAA CTC AAA CGA ACT GTG GCT GCA CCA TCT GTC TTC ATC TTC CCG CCA TCT GAT
lys val glu leu lys arg thr val ala ala pro ser val phe ile phe pro pro ser asp
121                                               131
GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT CCC AGA
glu gln leu lys ser gly thr ala ser val val cys leu leu asn asn phe tyr pro arg
141                                               151
GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC CAG GAG AGT
glu ala lys val gln trp lys val asp asn ala leu gln ser gly asn ser gln glu ser
161                                               171
GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC AGC ACC CTG ACG CTG AGC
val thr glu gln asp ser lys asp ser thr tyr ser leu ser ser thr leu thr leu ser
181                                               191
AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC ACC CAT CAG GGC CTG AGC
lys ala asp tyr glu lys his lys val tyr ala cys glu val thr his gln gly leu ser
201                                               211
TTG CCC GTC ACA AAG AGC TTC AAC AGG GGA GAG TGT TAG Ttc taq a
leu pro val thr lys ser phe asn arg gly glu cys AMB
```

Fig. 2B

10050593.120501

Method of grafting peptide into antibody with random sequences surrounding peptide sequence

```
                 FR3                        TPO Mimetic Peptide
(SEQ ID NO: 56 ) Y   Y   C   A   R   X   X   I   E   G   P   T   L   R   Q
(SEQ ID NO: 57 ) TATTAT-TGT-GCG-AGA-NNR-NNR-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-

                                             FR4
W   L   A   A   R   A   X   X   W   G   Q   G   T
TGG-CTG-GCG-GCG-CGC-GCG-NNY-NNY-TGG-GGC-CAA-GGG-ACC-
```

The TPO mimetic peptide was grafted into the heavy chain CDR3 region of the tetanus toxoid antibody. The peptide was flanked on either side by two random amino acids, shown as "X"s in the figure.

Fig. 3

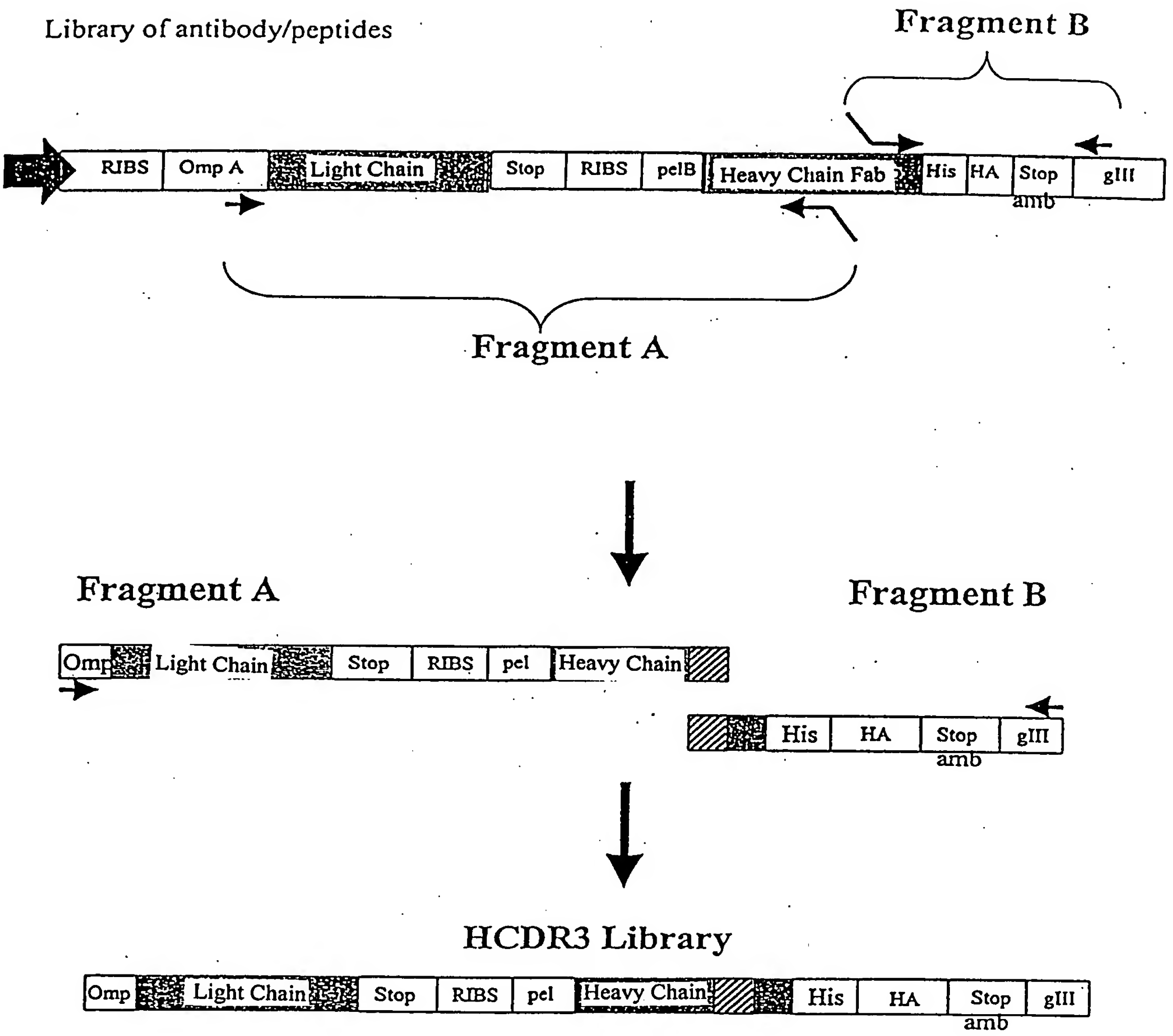
Library of antibody/peptides
Fragment B
RIBS
Omp A
Light Chain
Stop
RIBS
pelB
Heavy Chain Fab
His
HA
Stop
amb
gIII
Fragment A
Fragment A
Fragment B
Omp
Light Chain
Stop
RIBS
pel
Heavy Chain
His
HA
Stop
amb
gIII
HCDR3 Library
Omp
Light Chain
Stop
RIBS
pel
Heavy Chain
His
HA
Stop
amb
gIII

Fig. 4

10055923.120501

| CLONE | AMINO ACID SEQUENCE | SEQ ID NO. |
|---|---|---|
| X1a | Pro-Pro-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Gly-Gly<br>CCG-CCC-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-GGA-GGC | 25<br>26 |
| X1a-11 | Gly-Gly-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Gly-Gly<br>GGG-GGT-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-GGC-GGA | 27<br>28 |
| X1a-13 | Gly-Gly-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Gly-Gly<br>GGC-GGT-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-GGA-GGC | 29<br>30 |
| X1c | Trp-Leu-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val<br>TGG-CTG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCT-GTC | 31<br>32 |
| X2c | Met-Ile-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Val-Gly<br>ATG-ATA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-GTT-GGC | 33<br>34 |
| X3a | Val-Val-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val<br>GTG-GTA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCT-GTT | 35<br>36 |
| X3b | Gly-Pro-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Asp<br>GGG-CCG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCC-GAT | 37<br>38 |
| X4b | Leu-Pro-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val<br>TTG-CCA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCT-GTT | 39<br>40 |
| X4c | Ser-Leu-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Ile<br>TCA-CTG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCC-ATC | 41<br>42 |
| X5a | Thr-Met-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val<br>ACA-ATG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCC-GTT | 43<br>44 |
| X5c | Thr-Thr-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val<br>ACG-ACA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCT-GTC | 45<br>46 |
| X7a | Thr-Arg-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Cys-Ser<br>ACA-CGG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-TGC-AGC | 47<br>48 |
| X7b | no peptide deletion mutant | |
| X7c | Gln-Thr-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Asp<br>CAG-ACA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-CGC-GCG-CCT-CAC | 49<br>50 |

Fig. 5

10006593 . 120501

# pRL8

(SEQ ID NO: 60 )

GGGAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAA
ATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATC
AAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGT
CCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATC
AGGGCGATGGCCCACTACGTGAACCATCACCCTAATCAAGTTTTTTGGGGTC
GAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGA
GCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGC
GAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGT
AACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCAGGTGGC
ACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACA
TTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAAT
ATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCC
TTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAA
GTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGG
ATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCA
ATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGA
CGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTG
GTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAA
GAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTT
ACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAAC
ATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAG
CCATACCAAACGACGAGCTGTACACCACGATGCCTGTAGCAATGGCAACAAC
GTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAAT
TAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGC
CCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGT
CTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGT
AGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACA
GATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAA
GTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGG
ATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGA
GTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTT
GAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCG
CTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAA
GGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAG
CCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGC
TCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTA
CCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTG
AACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGA

Fig. 6A

10005593.120501

ACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGG
AGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGC
ACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGT
TTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGG
AGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTG
CTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAA
CCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACC
GAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAA
CCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTT
TCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTC
ACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTG
TGGAATTGTGAGCGGATAACAATTGAATTCAGGAGGAATTTAAAATGAAAAA
GACAGCTATCGCGATTGCAGTGGCACTGGCTGGTTTCGCTACCGTGGCCCAG
GCGGCCGAGCTCGGCCATGGCTGGTTGGGCAGCGAGTAATAACAATCCAGCG
GCTGCCGTAGGCAATAGGTATTTCATTATGACTGTCTCCTTGGCGACTAGCTA
GTTTAGAATTCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCC
GCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGG
GGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGC
TTTCCAGTCGGGAAACCTGTCGTGTTACTAATGATGGTGATGGTGATGGCTAG
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TGTGATTCACGTTGCAGATGTAGGTCTGGGTGCCCAAGCTGCTGGAGGGCAC
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TTCgGGGAAGTAGTCCTTGACCAGGCAGCCCAGGGCCGCTGTGCCCCCAGAG
GTGCTCTTGGAGGAGGGTGCCAGGGGGAAGACCGATGGGCCCTTGGTGGAG
GCTGCGGAGACGGTGACCGTGGTACCAGCAGAAACCTGGCCAGGCTCCCAG
GCTCCTCATCTATGGTACATCCAGCAGGGCCACTGGCATCCCAGACAGGTTC
AGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGC
CTGAAGATTTTGCAGTGTACTACTGTCAGCAGTATGGTGGCTCACCGTGGTTC
GGCCAAGGGACCAAGGTGGAACTCAAACGAACTGTGGCTGCACCATCTGTCT
TCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTG
TGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGG
ATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAG
CAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGA
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ATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCAG
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ACGTTCCGGACTACGGTTCTACTAGTccgaaaccgtctaccccaccgggctcttcctgcggtggccgc
atcgcccgtctggaggaaaaagtgaaaaccctgaaagctcagaactccgagctggcgtccactgccaacatgctgcgcgaac

Fig. 6B

aggtggcacagctgaaacagaaagttatgaaccatggcggttgtgctagtGGCCAGGCCGGCCAGCACCAT
CACCATCACCATGGCGCATACCCGTACGACGTTCCGGACTACGCTTCTTAGG
AGGGTGGTGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGAGGGAGG
CGGTTCCGGTGGTGGCTCTGGTTCCGGTGATTTTGATTATGAAAAGATGGCAA
ACGCTAATAAGGGGGCTATGACCGAAAATGCCGATGAAAACGCGCTACAGTC
TGACGCTAAAGGCAAACTTGATTCTGTCGCTACTGATTACGGTGCTGCTATCG
ATGGTTTCATTGGTGACGTTTCCGGCCTTGCTAATGGTAATGGTGCTACTGGT
GATTTTGCTGGCTCTAATTCCCAAATGGCTCAAGTCGGTGACGGTGATAATTC
ACCTTTAATGAATAATTTCCGTCAATATTTACCTTCCCTCCCTCAATCGGTTGA
ATGTCGCCCTTTTGTCTTTAGCGCTGGTAAACCATATGAATTTTCTATTGATTG
TGACAAAATAAACTTATTCCGTGGTGTCTTTGCGTTTCTTTTATATGTTGCCAC
CTTTATGTATGTATTTTCTACGTTTGCTAACATACTGCGTAATAAGGAGTCTTA
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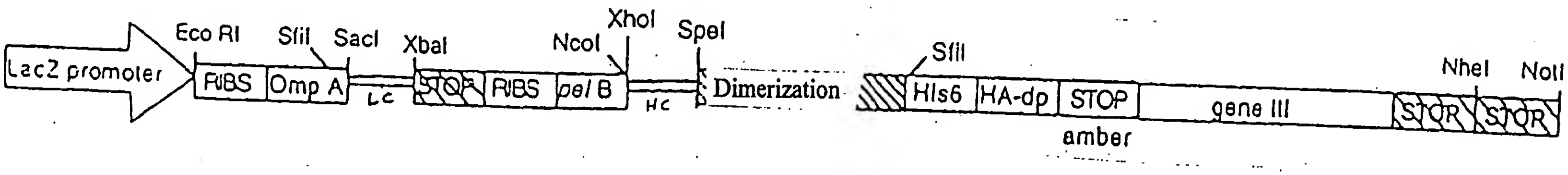
Fig. 6C

10065593.120501

pRL8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RIBS
SfiI
Omp A
SacI
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XbaI
STOP
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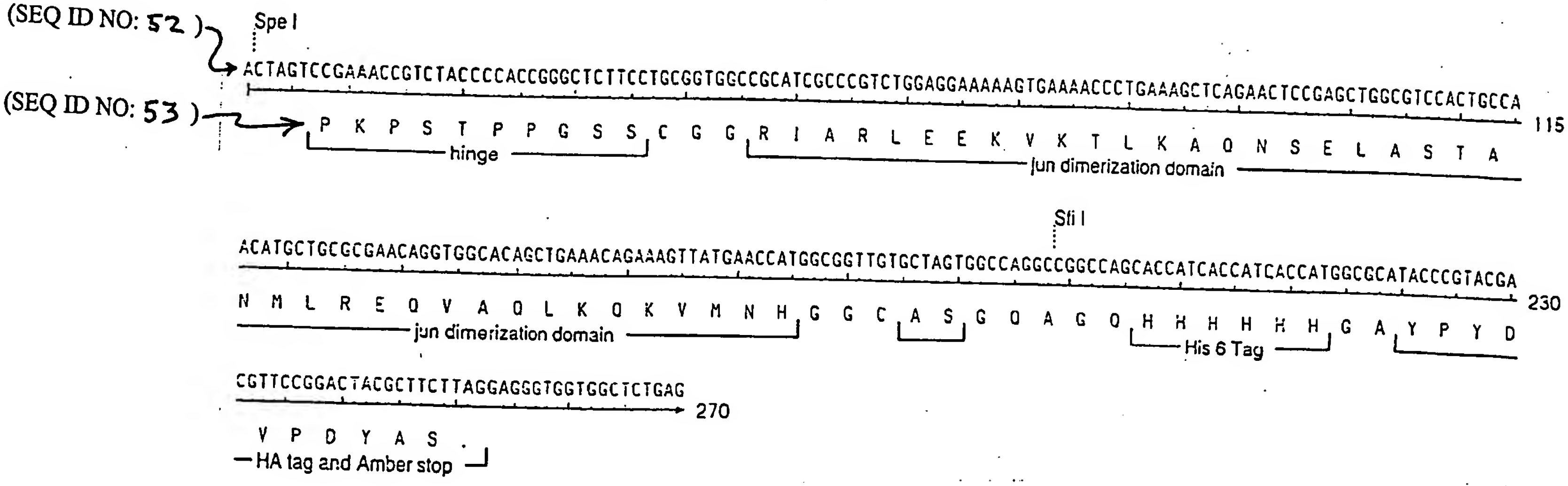
NcoI
pel B
XhoI
HC
SpeI
Dimerization
SfiI
His6
HA-dp
STOP
amber
gene III
NheI
STOP
NotI
STOP

Fig. 7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(SEQ ID NO: 53)
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115
P K P S T P P G S S C G G R I A R L E E K V K T L K A Q N S E L A S T A
hinge
jun dimerization domain
Sfi I
ACATGCTGCGCGAACAGGTGGCACAGCTGAAACAGAAAGTTATGAACCATGGCGGTTGTGCTAGTGGCCAGGCCGGCCAGCACCATCACCATCACCATGGCGCATACCCGTACGA
230
N M L R E Q V A Q L K Q K V M N H G G C A S G Q A G Q H H H H H H G A Y P Y D
jun dimerization domain
His 6 Tag
CGTTCCGGACTACGCTTCTTAGGAGGGTGGTGGCTCTGAG
270
V P D Y A S .
HA tag and Amber stop

Fig. 8

TPO Positive Clones nnk nnk 1 2 IEGPTLRQWLAARA nnk nnk 3 4

| | Sample | nnk nnk | nnk nnk | Amino Acids | Amino Acids |
|---|---|---|---|---|---|
| | | 1 2 | 3 4 | 1 2 | 3 4 |
| HC CDR3 | X1c | tgg ctg | cct gtc | Trp Leu | Pro Val |
| | X3a | gtg gta | cct gtt | Val Val | Pro Val |
| | X3b | ggg ccg | ccc gat | Gly Pro | Pro Asp |
| | X4b | ttg cca | cct gtt | Leu Pro | Pro Val |
| | X4c | tca ctg | ccc atc | Ser Leu | Pro Ile |
| | X5a | aca atg | ccc gtt | Thr Met | Pro Val |
| | X5c | acg aca | cct gtc | Trp Leu | Pro Val |
| | X7c | cag aca | cct cac | Gln Thr | Pro Asp |
| HC CDR2 | 24 | ctt tat | tct aat | Leu Tyr | Ser Asn |
| | 39 | act tac | ttg cat | Thr Tyr | Leu His |
| | 3 | agg atg | ctc gag | Arg Met | Leu Glu |
| | 7 | aag gaa | tct aag | Lys Glu | Ser Lys |
| | 8 | gcg cat | gtg cag | Ala His | Val Gln |
| | 10 | cag gag | att agt | Gln Glu | Ile Ser |
| | 11 | cgg aat | aat ccg | Arg Asn | Asn Pro |
| | 19 | cag cta | aat tct | Gln Leu | Asn Ser |
| | 25 | agt att | ttt gtc | Ser Ile | Phe Val |
| | 28 | ggg ccc | act agt | Gly Pro | Thr Ser |
| LC CDR1 | 10 | aag ggt | gtt agt | Lys Gly | Val Ser |
| | 11 | cat ggg | gtg gct | His Gly | Val Ala |
| | 12a | cgt acg | atg gct | Arg Thr | Met Ala |
| | 12b | cgt ggt | gtt aat | Arg Gly | Val Asn |
| | 14 | cgt tcg | ctt gcg | Arg Ser | Leu Ala |
| | 16 | cgg ggt | gtt gcg | Arg Gly | Val Ala |
| | 18 | agg acg | gtg tct | Arg Thr | Val Ser |
| | 47 | aag ggg | gtg gcg | Lys Gly | Val Ala |
| LC CDR2 | 1 | aat ccg | agg ggt | Asn Pro | Arg Gly |
| | 2 | tcg cct | cgg agt | Ser Pro | Arg Ser |
| | 3 | tcg cct | cgt acg | Ser Pro | Arg Thr |
| | 4 | tcg cct | tgg cgt | Ser Pro | Trp Arg |
| | 5 | act ccg | aat tgg | Thr Pro | Asn Trp |
| | 6 | aat cct | gcg agg | Asn Pro | Ala Arg |
| | 7 | aat ccg | tcg ggg | Asn Pro | Ser Gly |
| | 9 | aat cct | tat tag | Asn Pro | Tyr Stop |
| | 10 | aat ccg | cgg tct | Asn Pro | Arg Ser |
| | 11 | aat ccg | gat gtg | Asn Pro | Asp Val |
| | 12 | tcg ccg | tcg cgg | Ser Pro | Ser Arg |
| | 13 | aat cct | ctg ttt | Asn Pro | Leu Phe |
| | 14 | aat ctt | ggg tat | Asn Pro | Gly Tyr |
| | 15 | aat cct | att agt | Asn Pro | Ile Ser |
| | 16 | aat cct | cag cgg | Asn Pro | Gln Arg |
| | 18 | aat ccg | cgg acg | Asn Pro | Arg Thr |
| | 19 | aat ccg | cgt ggg | Asn Pro | Arg Gly |
| | 20 | cat ttg | aga ctg | His Leu | Arg Leu |
| | 21 | aag tag | att tat | Lys Stop | Ile Tyr |
| | 23 | aat cct | ggt aag | Asn Pro | Gly Lys |
| | 24 | aat cct | cgt ggg | Asn Pro | Arg Gly |
| | 26 | aat cct | aat gtg | Asn Pro | Asn Val |
| | 27 | tct ccg | cgg gtt | Ser Pro | Arg Val |
| | 29 | acg cct | cgg ggt | Thr Pro | Arg Gly |
| | 30 | cct tag | tgg tgg | Pro Stop | Trp Trp |

FIG. 9

10006593.120501

Activity of Fab clones containing 2 TPO mimetic peptides

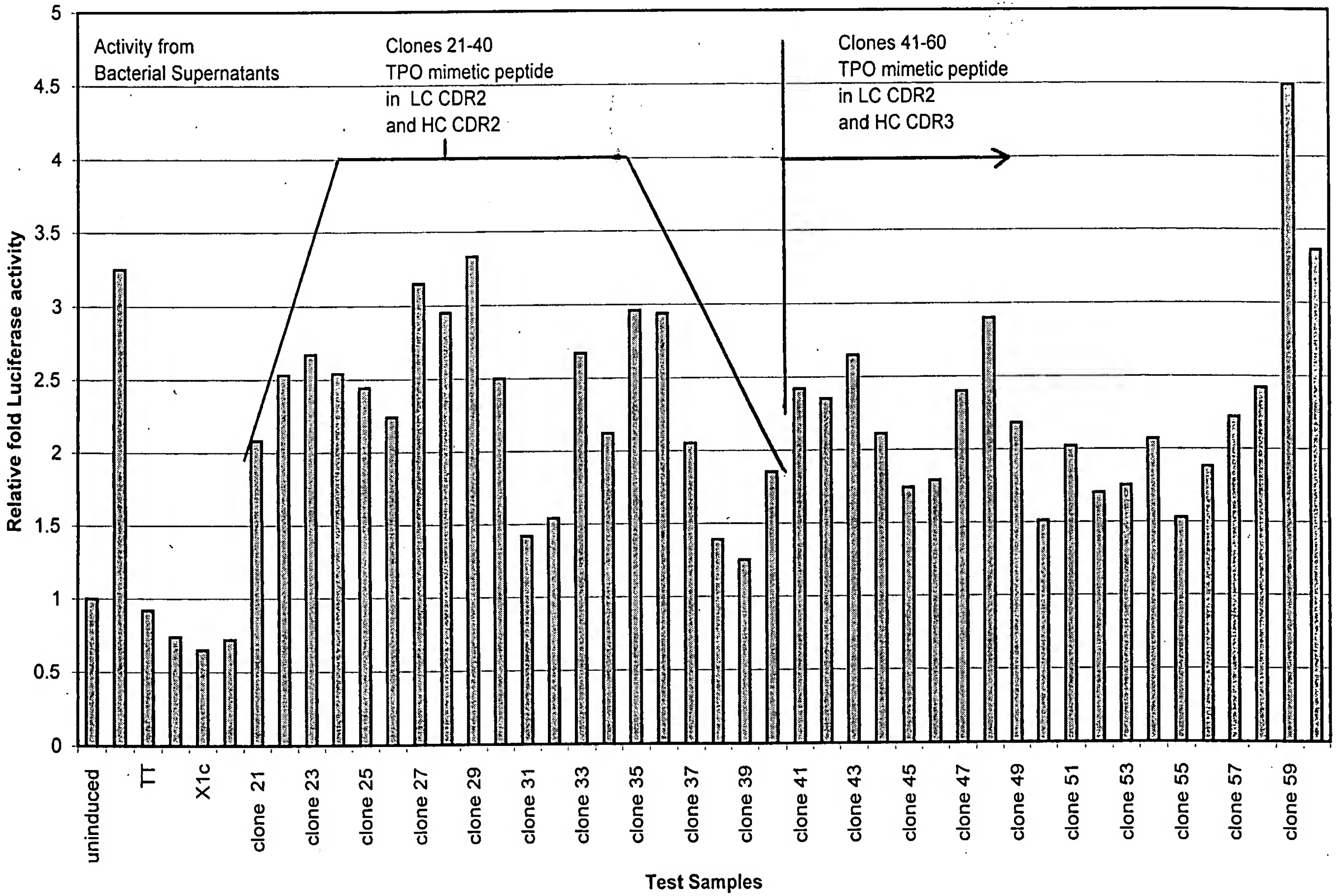


FIGURE 10

Activity of Fab clones containing 2 or 3 TPO mimetic peptides

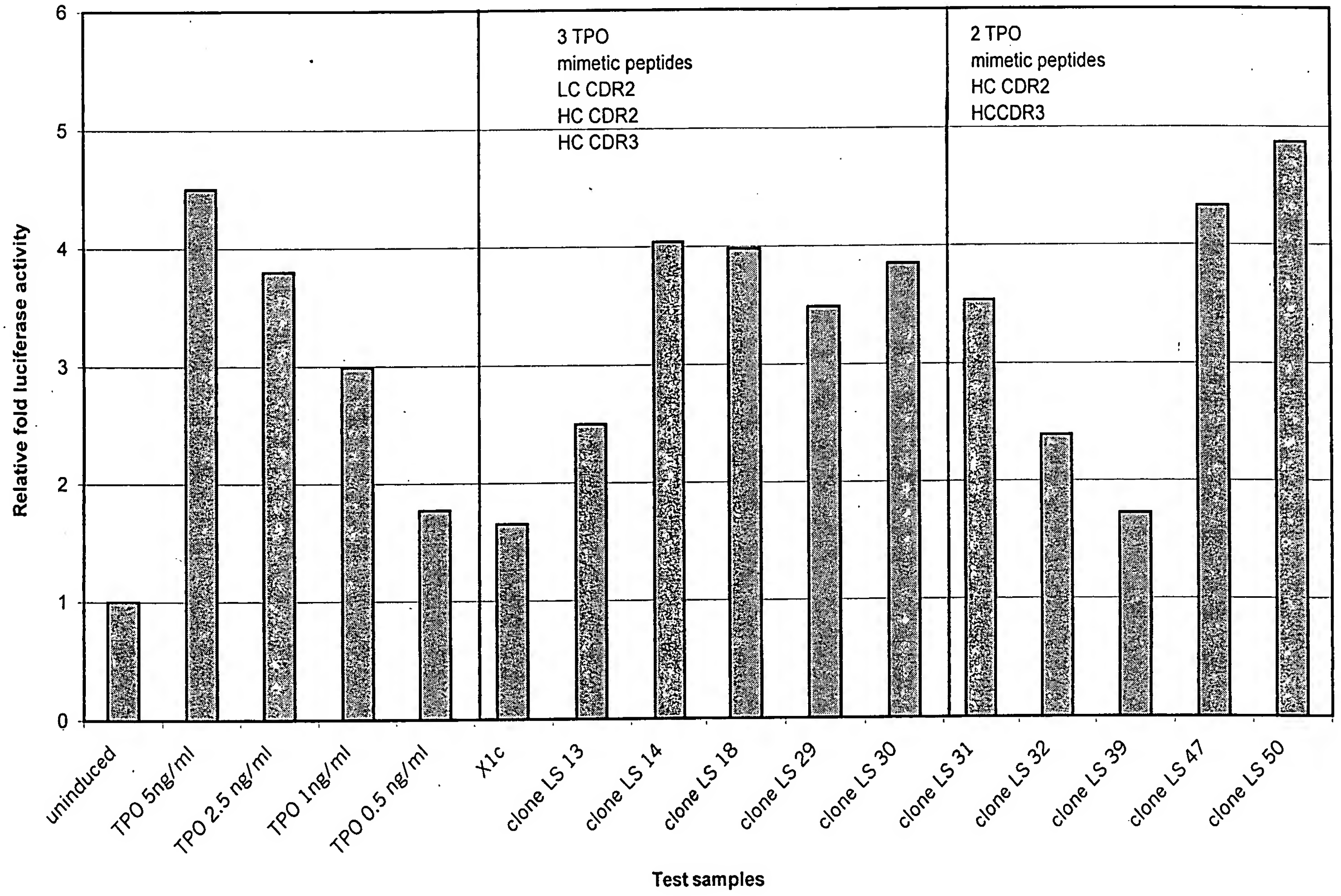


FIGURE 11

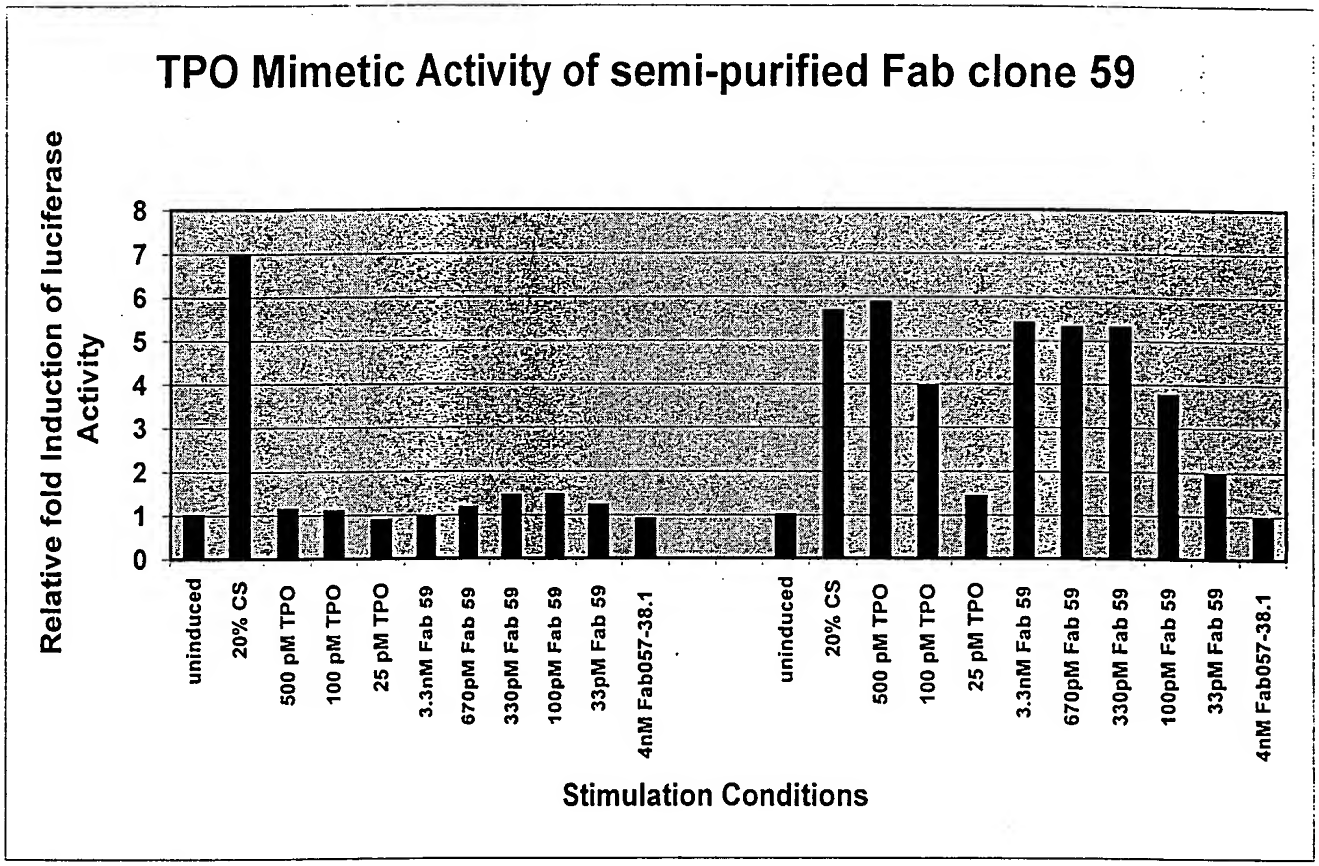

TPO Mimetic Activity of semi-purified Fab clone 59

Fig. 12

10065593.120501

(SEQ ID NO: 67 )

5G1.1 – TPO Heavy Chain (Bold denotes TPO mimetic) Amino acid sequence:
*MKIVSWVILFLLSVTAGVHS*QVQLVQSGAEVKKPGASVKVSCKASGYIFSNYWIQW
VRQAPGQGLEWMGEILPGSGSTEYTENFKDRVTMTRDTSTSTVYMELSSLRSED
TAVYYCAR**LPIEGPTLRQWLAARA**PVWGQGTLVTVSSASTKGPSVFPLAPCSR
STSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV
PSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRL
TVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK.

(SEQ ID NO: 68 )

5G1.1 – TPO Heavy Chain (Bold denotes TPO mimetic) Nucleic acid sequence:
ATGAAGTGGAGCTGGGTTATTCTCTTCCTCCTGTCAGTAACTGCCGGCGTCCA
CTCCCAAGTCCAACTGGTGCAATCCGGCGCCGAGGTCAAGAAGCCAGGGGCC
TCAGTCAAAGTGTCCTGTAAAGCTAGCGGCTATATTTTTTCTAATTATTGGAT
TCAATGGGTGCGTCAGGCCCCCGGGCAGGGCCTGGAATGGATGGGTGAGATC
TTACCGGGCTCTGGTAGCACCGAATATACCGAAAATTTTAAAGACCGTGTTA
CTATGACGCGTGACACTTCGACTAGTACAGTATACATGGAGCTCTCCAGCCTG
CGATCGGAGGACACGGCCGTCTATTATTGCGCGCGTTTGCCAATTGAAGGG
**CCGACGCTGCGGCAATGGCTGGCGGCGCGCGCGCCTGTTTGGGGTCAAG**
GAACCCTGGTCACTGTCTCGAGCGCCTCCACCAAGGGCCCATCCGTCTTCCCC
CTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGCCCTGGGCTGCC
TGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGC
CCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCT
ACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGAC
CTACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGAC
AGTTGAGCGCAAATGTTGTGTCGAGTGCCCACCGTGCCCAGCACCACCTGTG
GCAGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGAT
CTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGCCAGGAAGAC
CCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCA
AGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCG
TCCTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAA
GGTCTCCAACAAAGGCCTCCCGTCCTCCATCGAGAAAACCATCTCCAAAGCC
AAAGGGCAGCCCCGAGAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAG
GAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACC
CCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACT
ACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGC
AGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGCT
CCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCT
GTCTCTGGGTAAATGA

Fig. 13A

(SEQ ID NO: 69 )

5G1.1 Light Chain Amino Acid Sequence

*MDMRVPAQLLGLLLLWLRGARC*DIQMTQSPSSLSASVGDRVTITCGASENIYGALN
WYQQKPGKAPKLLIYGATNLADGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ
NVLNTPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC.

(SEQ ID NO: 70 )

5G1.1 Light Chain Nucleic Acid Sequence

ATGGACATGAGGGTCCCCGCTCAGCTCCTGGGGCTCCTGCTACTCTGGCTCCG
AGGTGCCAGATGTGATATCCAGATGACCCAGTCCCCGTCCTCCCTGTCCGCCT
CTGTGGGCGATAGGGTCACCATCACCTGCGGCGCCAGCGAAAACATCTATGG
CGCGCTGAACTGGTATCAACAGAAACCCGGGAAAGCTCCGAAGCTTCTGATT
TACGGTGCGACGAACCTGGCAGATGGAGTCCCTTCTCGCTTCTCTGGATCCGG
CTCCGGAACGGATTTCACTCTGACCATCAGCAGTCTGCAGCCTGAAGACTTC
GCTACGTATTACTGTCAGAACGTTTTAAATACTCCGTTGACTTTCGGACAGGG
TACCAAGGTGGAAATAAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCC
CGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTG
AATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCC
TCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACA
GCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGA
AACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGT
CACAAAGAGCTTCAACAGGGGAGAGTGTTAG

Note: Italics denotes leader sequence

Fig. 13B

FACS staining on transfected 293 cells

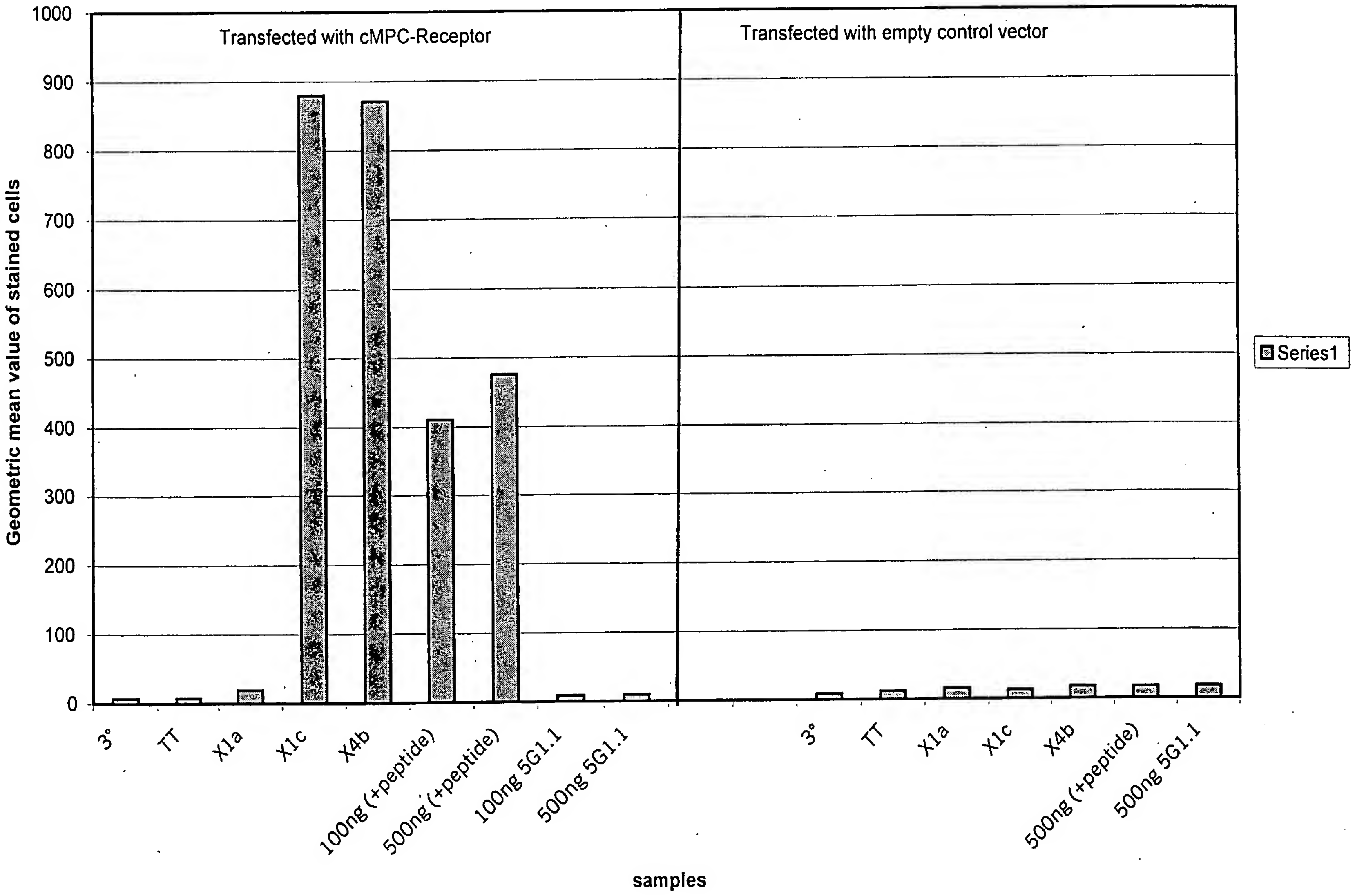


FIGURE 14

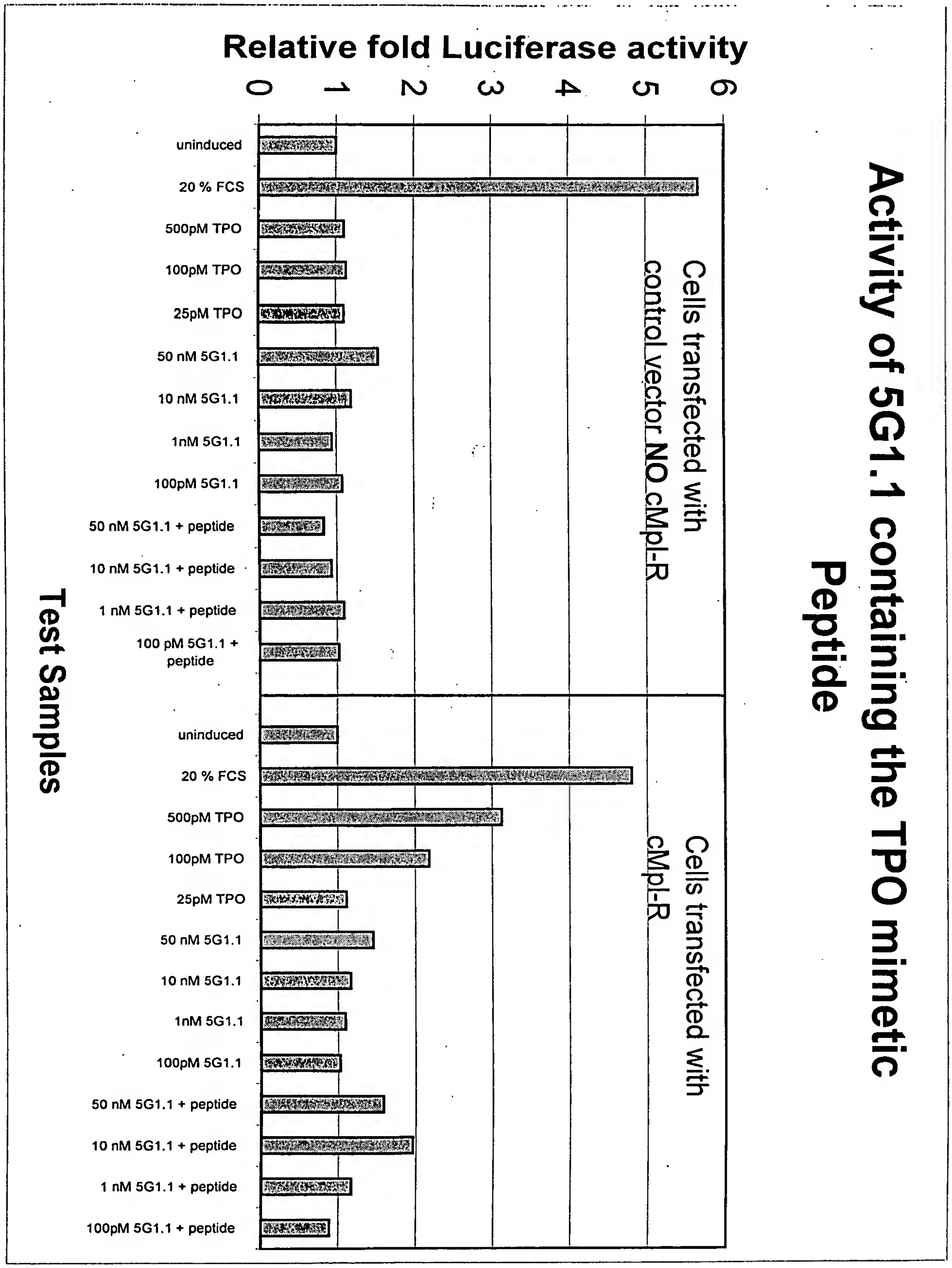

Activity of 5G1.1 containing the TPO mimetic Peptide
Relative fold Luciferase activity
0
1
2
3
4
5
6
Cells transfected with control vector NO cMpl-R
Cells transfected with cMpl-R
uninduced
20 % FCS
500pM TPO
100pM TPO
25pM TPO
50 nM 5G1.1
10 nM 5G1.1
1nM 5G1.1
100pM 5G1.1
50 nM 5G1.1 + peptide
10 nM 5G1.1 + peptide
1 nM 5G1.1 + peptide
100 pM 5G1.1 + peptide
uninduced
20 % FCS
500pM TPO
100pM TPO
25pM TPO
50 nM 5G1.1
10 nM 5G1.1
1nM 5G1.1
100pM 5G1.1
50 nM 5G1.1 + peptide
10 nM 5G1.1 + peptide
1 nM 5G1.1 + peptide
100pM 5G1.1 + peptide
Test Samples

**FIGURE 15**

VARIABLE REGION OF 4-29 LIGHT CHAIN

(SEQ ID NO: 116)

CDR1 (24–28)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 27a 27b 27c 27d 27e 27f 28
GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC CGG GCG AGT CAG ... ... ... ... ... ...AGT

CDR1 (29–34) · Grafted CDR2 (50–52j)

29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 52a 52b 52c 52d 52e 52f 52g 52h 52i 52j
ATT AGT AGT TTG CTG GCC TGG TAT CAG CAG AAA CCA GGG AAA GCT CCT AAG CTC CTG ATC TAT AAC CCG ATC GAA GGC CCA ACC CTG CGC CAG TGG CTG GCT

Grafted CDR2 (52k–56)

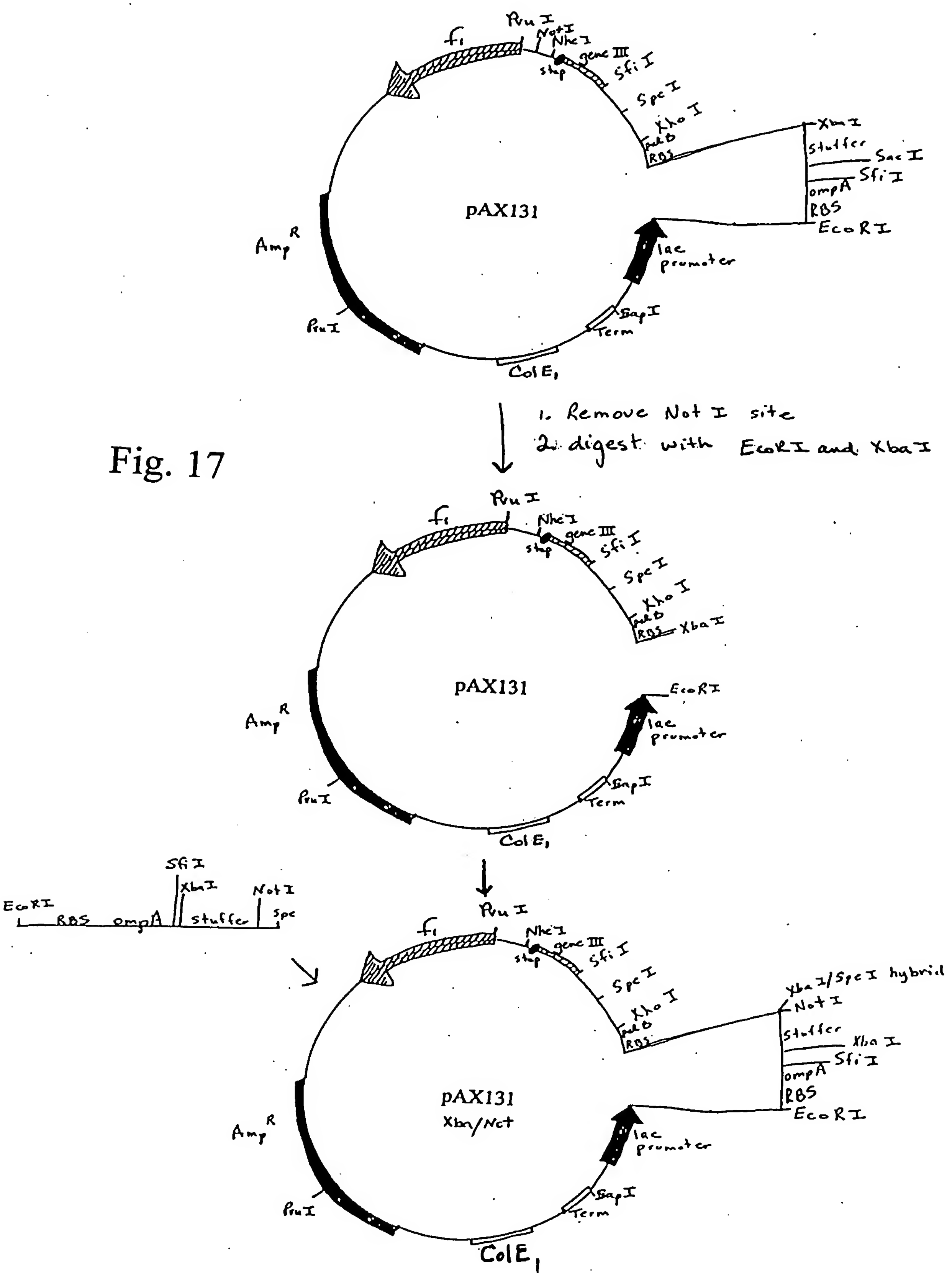
52k 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85
ACT CGC GCT CGT GGT GGG GTC CCA TCA AGG TTC AGC GGC AGT GGA TCT GGG ACA GAT TTC ACT CTC ACC ATC AGC AGC CTG CAG CCT GAA GAT TTT GCA ACT

CDR3 (89–97)

86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107
TAT TAC TGC CAA CAG TAT AAT AGT TAC CCT CCC ACT TTC GGC CCT GGG ACC AAA GTG GAT ATC AAA

Fig. 16

Fig. 17


f1
PvuI
NotI
NheI
gene III
stop
SfiI
SpeI
XhoI
pelB
RBS
XbaI
stuffer
SacI
SfiI
ompA
RBS
EcoRI
pAX131
lac promoter
AmpR
PvuI
SapI
Term
ColE1
f1
PvuI
NheI
gene III
stop
SfiI
SpeI
XhoI
pelB
RBS
XbaI
EcoRI
pAX131
lac promoter
AmpR
PvuI
SapI
Term
ColE1
EcoRI
RBS
ompA
SfiI
XbaI
stuffer
NotI
Spe
f1
PvuI
NheI
gene III
stop
SfiI
SpeI
XhoI
pelB
RBS
XbaI/SpeI hybrid
NotI
stuffer
XbaI
SfiI
ompA
RBS
EcoRI
pAX131
Xba/Not
lac promoter
AmpR
PvuI
SapI
Term
ColE1

1. Remove NotI site
2. digest with EcoRI and XbaI

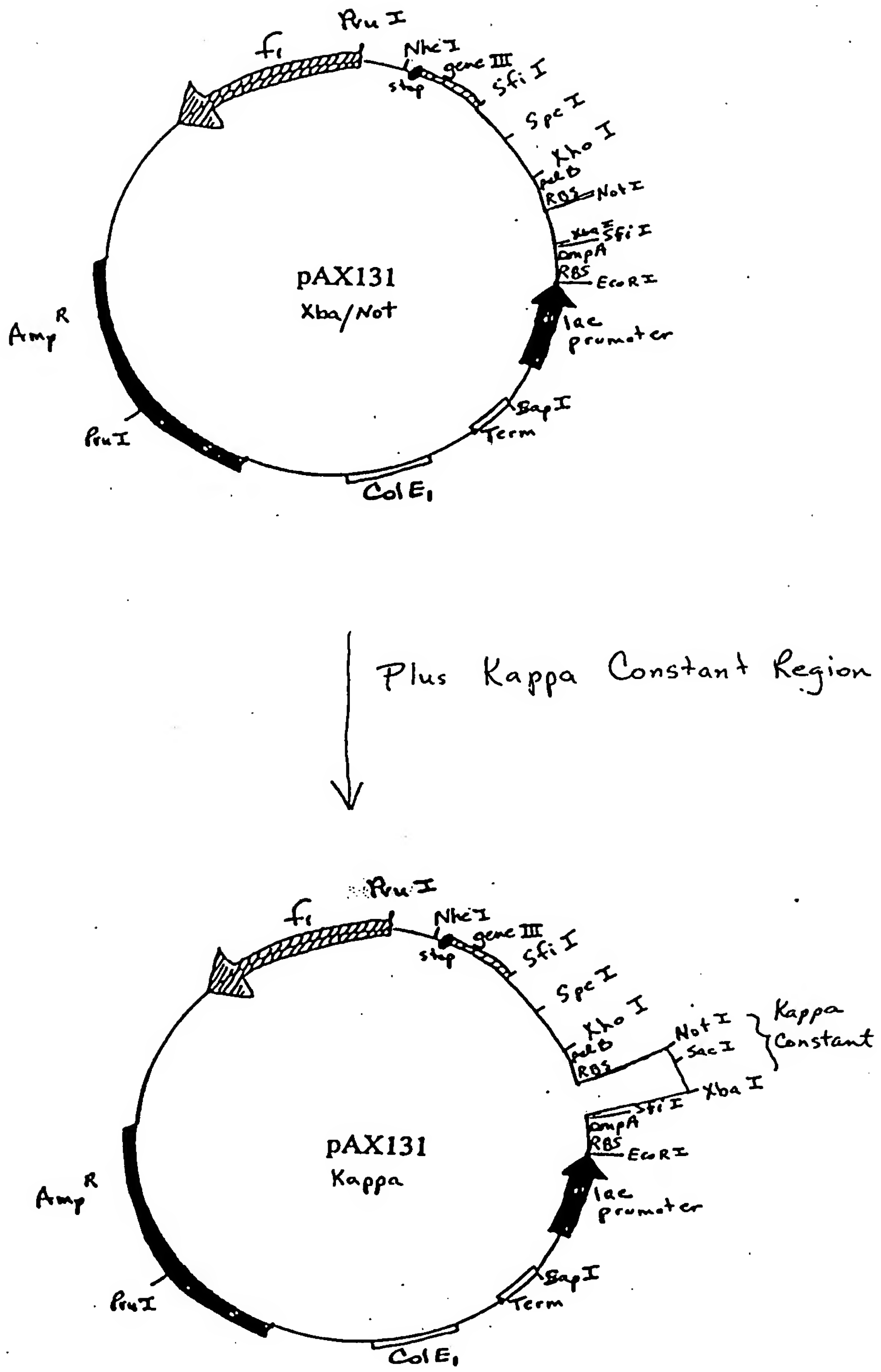

Pru I
f1
Nhe I
gene III
stop
Sfi I
Spe I
Xho I
pelB
RBS
Not I
Xba I
Sfi I
ompA
RBS
EcoR I
lac promoter
pAX131
Xba/Not
AmpR
Pru I
Sap I
Term
Col E1
Plus Kappa Constant Region
Pru I
f1
Nhe I
gene III
stop
Sfi I
Spe I
Xho I
pelB
RBS
Not I
Sac I
Kappa Constant
Xba I
Sfi I
ompA
RBS
EcoR I
lac promoter
pAX131
Kappa
AmpR
Pru I
Sap I
Term
Col E1

Fig. 18

3

10065593.120501

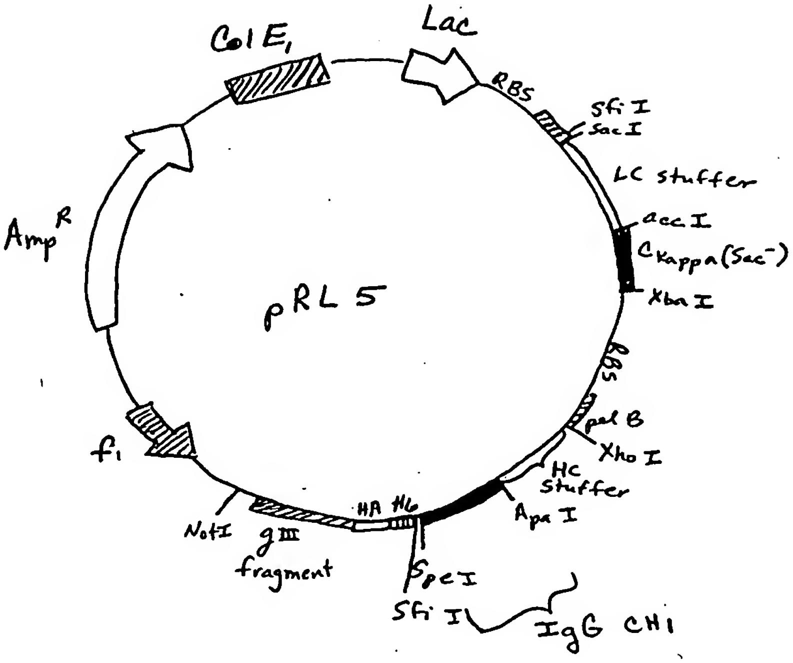
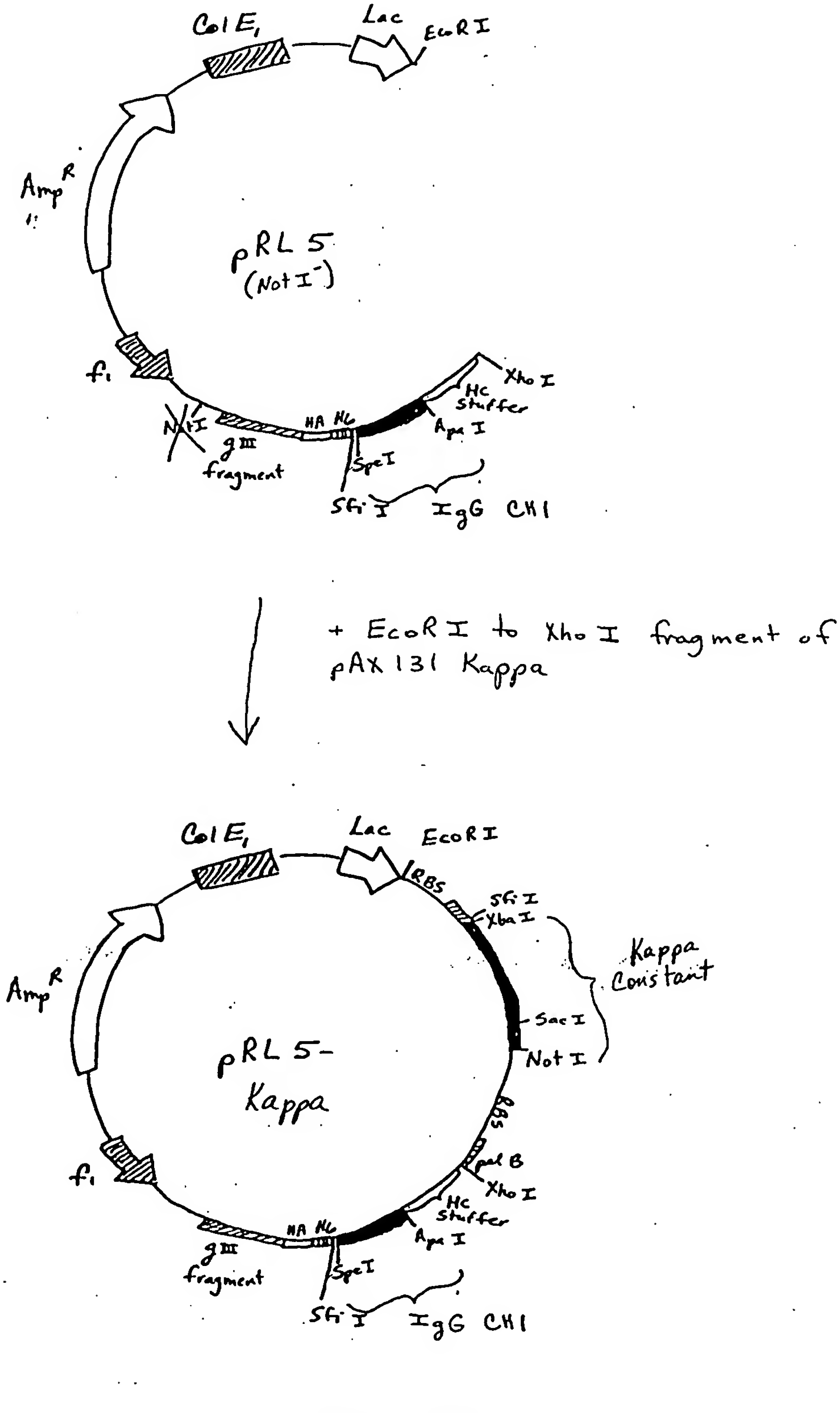

Col E1
Lac
RBS
Sfi I
Sac I
LC stuffer
acc I
Ckappa (Sac-)
Xba I
RBS
pel B
Xho I
HC stuffer
Apa I
IgG CH1
Spe I
Sfi I
H6
HA
gIII fragment
Not I
f1
AmpR
pRL5

Fig. 19

10065593 . 120501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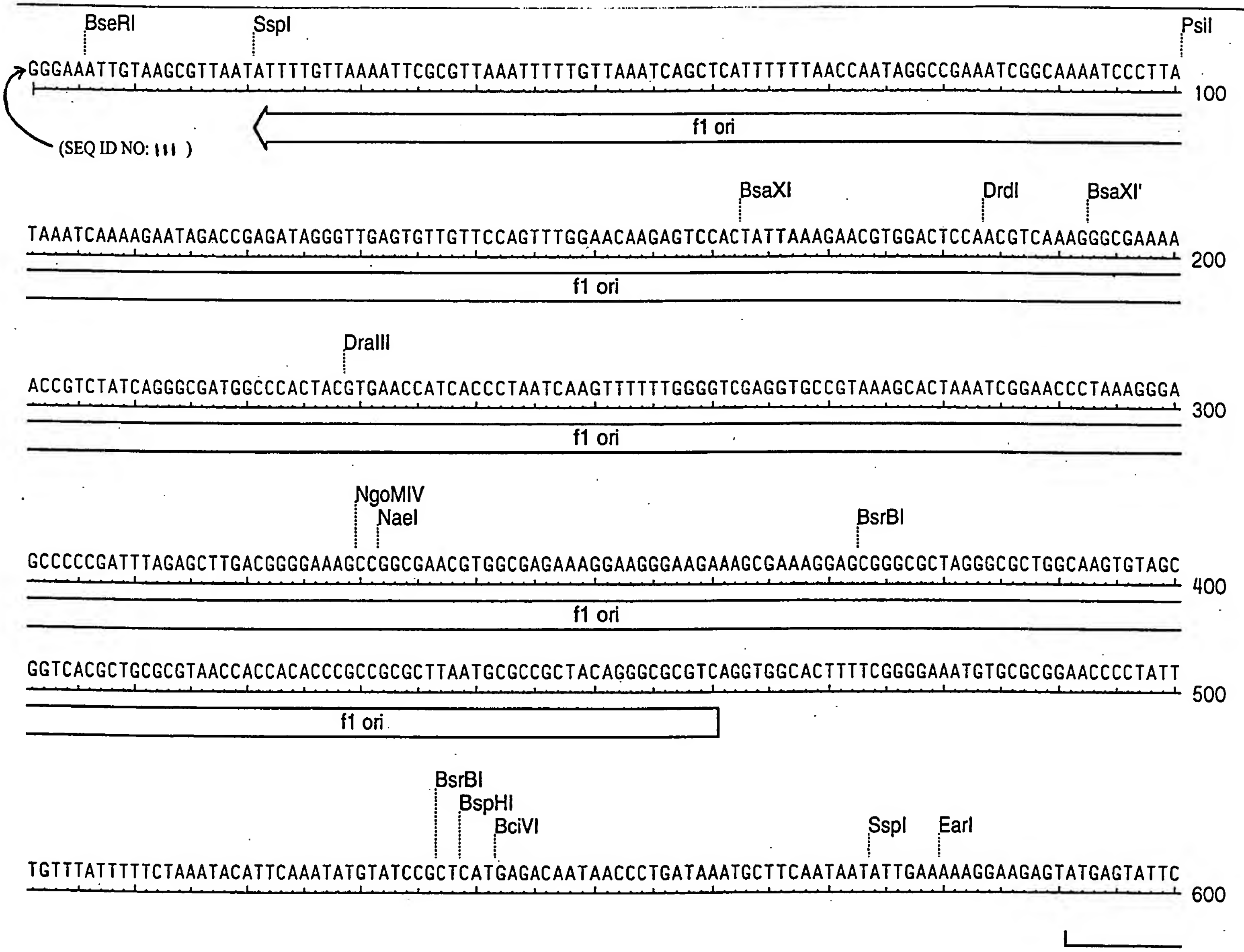
Lac
EcoRI
AmpR
pRL5 (NotI⁻)
f1
NotI
gIII fragment
HA
H6
SpeI
SfiI
IgG CH1
ApaI
HC Stuffer
XhoI
+ EcoRI to XhoI fragment of pAX131 Kappa
ColE1
Lac
EcoRI
RBS
SfiI
XbaI
Kappa Constant
SacI
NotI
RBS
pelB
XhoI
HC Stuffer
ApaI
AmpR
pRL5-Kappa
f1
gIII fragment
HA
H6
SpeI
SfiI
IgG CH1

Fig. 20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SspI
PsiI
(SEQ ID NO: 111 )
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BsaXI
DrdI
BsaXI'
f1 ori
DraIII
f1 ori
NgoMIV
NaeI
BsrBI
f1 ori
f1 ori
BsrBI
BspHI
BciVI
SspI
EarI

GGGAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTA 100

TAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAA 200

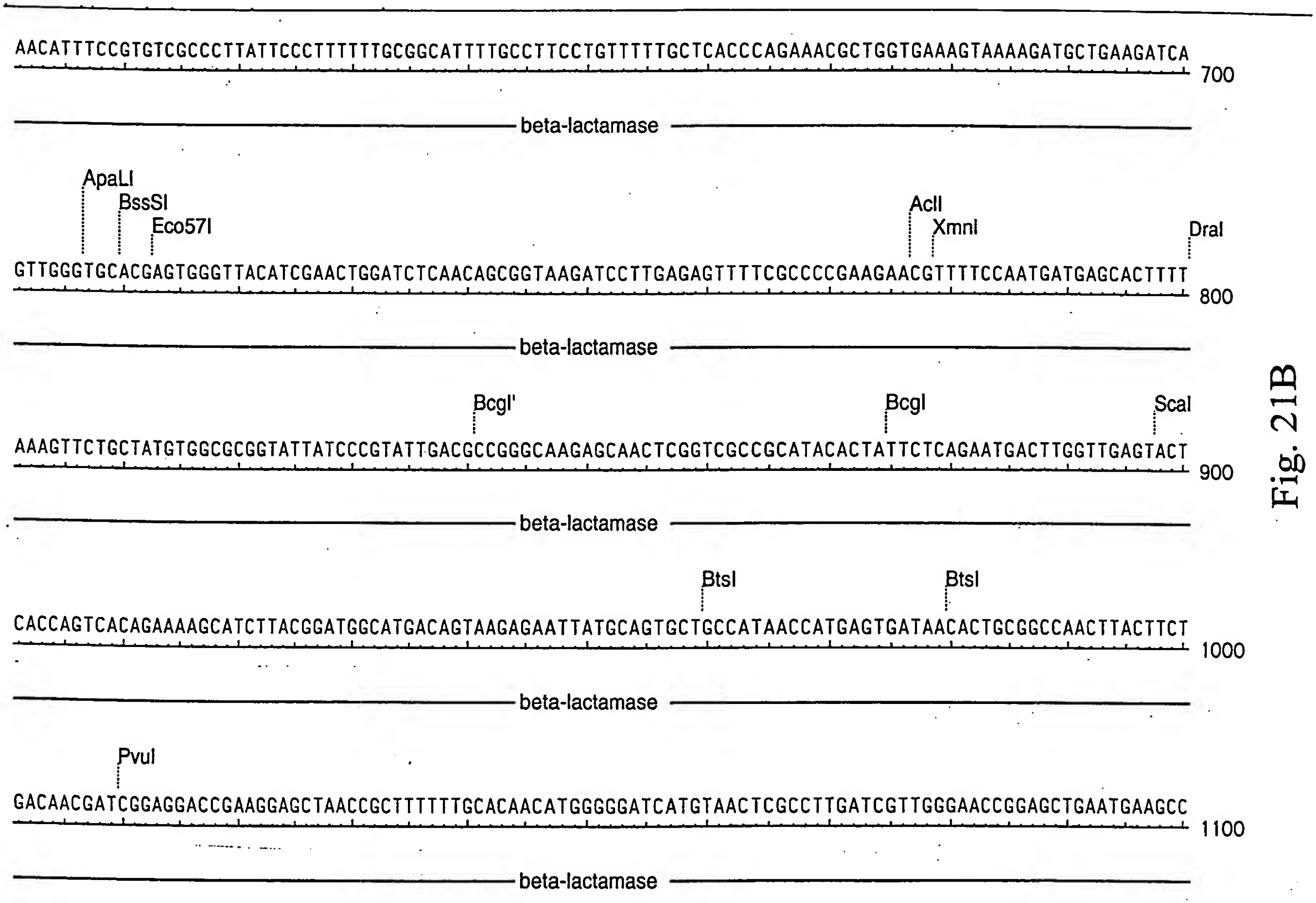
ACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGA 300

GCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGC 400

GGTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATT 500

TGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTC 600

Fig. 21A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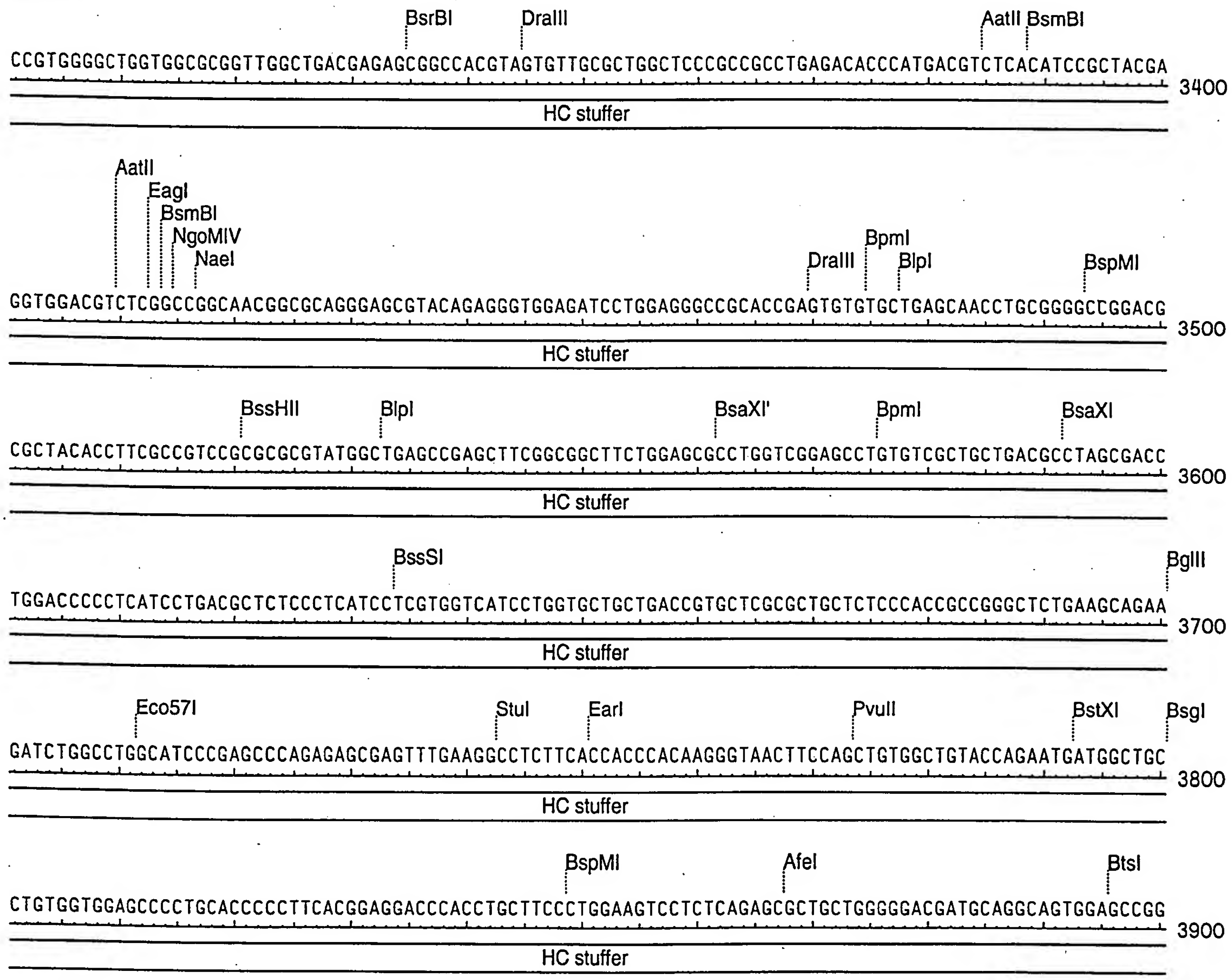
BssSI
Eco57I
AclI
XmnI
DraI
GTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTT
800
beta-lactamase
BcgI'
BcgI
ScaI
AAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACT
900
beta-lactamase
BtsI
BtsI
CACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCT
1000
beta-lactamase
PvuI
GACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCC
1100
beta-lactamase

Fig. 21B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DraIII
AatII BsmBI
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3400
HC stuffer
AatII
EagI
BsmBI
NgoMIV
NaeI
DraIII
BpmI
BlpI
BspMI
GGTGGACGTCTCGGCCGGCAACGGCGCAGGGAGCGTACAGAGGGTGGAGATCCTGGAGGGCCGCACCGAGTGTGTGCTGAGCAACCTGCGGGGCCGGACG
3500
HC stuffer
BssHII
BlpI
BsaXI'
BpmI
BsaXI
CGCTACACCTTCGCCGTCCGCGCGTATGGCTGAGCCGAGCTTCGGCGGCTTCTGGAGCGCCTGGTCGGAGCCTGTGTCGCTGCTGACGCCTAGCGACC
3600
HC stuffer
BssSI
BglII
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3700
HC stuffer
Eco57I
StuI
EarI
PvuII
BstXI
BsgI
GATCTGGCCTGGCATCCCGAGCCCAGAGAGCGAGTTTGAAGGCCTCTTCACCACCCACAAGGGTAACTTCCAGCTGTGGCTGTACCAGAATGATGGCTGC
3800
HC stuffer
BspMI
AfeI
BtsI
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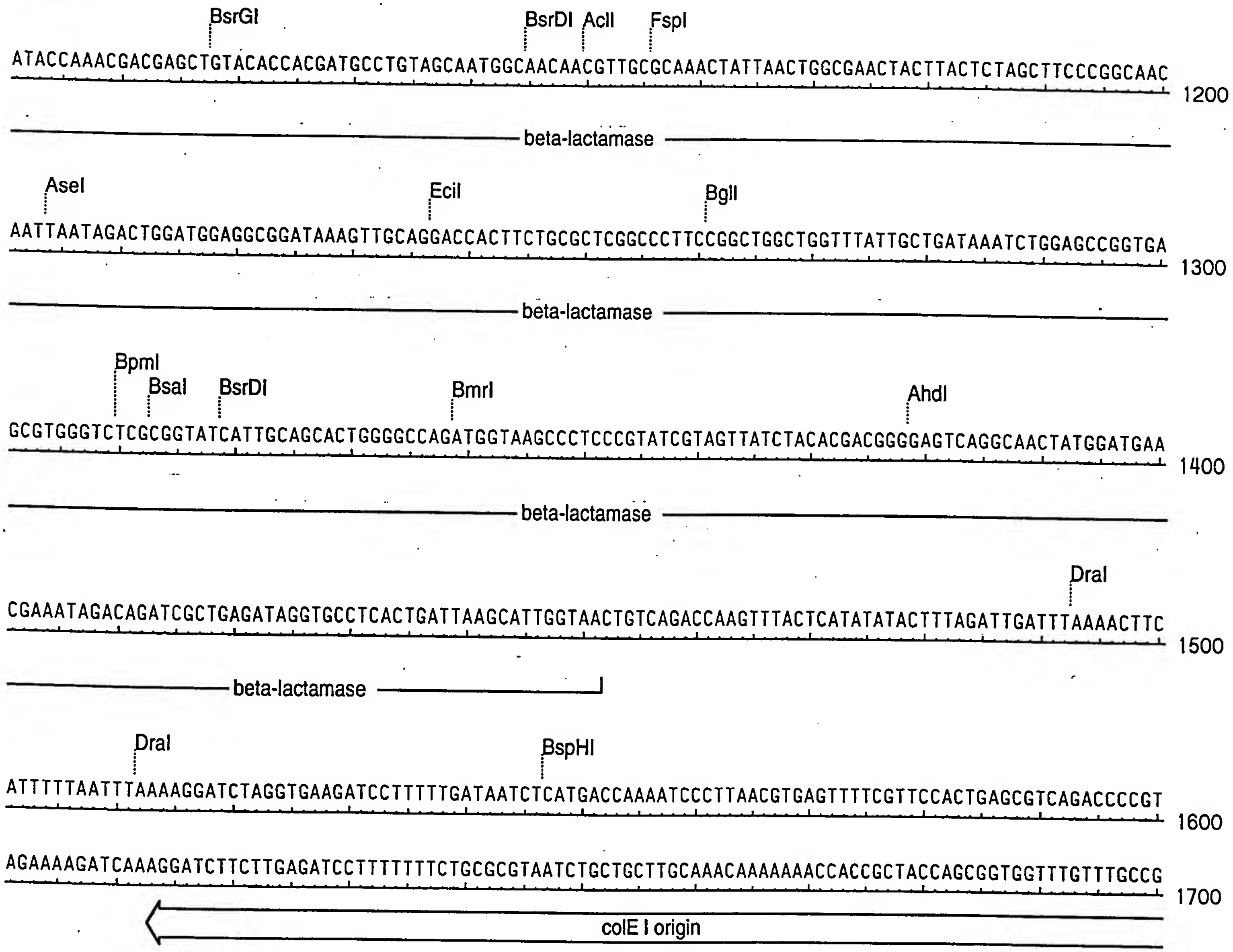
3900
HC stuffer

Fig. 21C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BsrDI
AclI
FspI
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1200
beta-lactamase
AseI
EciI
BglI
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1300
beta-lactamase
BpmI
BsaI
BsrDI
BmrI
AhdI
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1400
beta-lactamase
DraI
CGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTC
1500
beta-lactamase
DraI
BspHI
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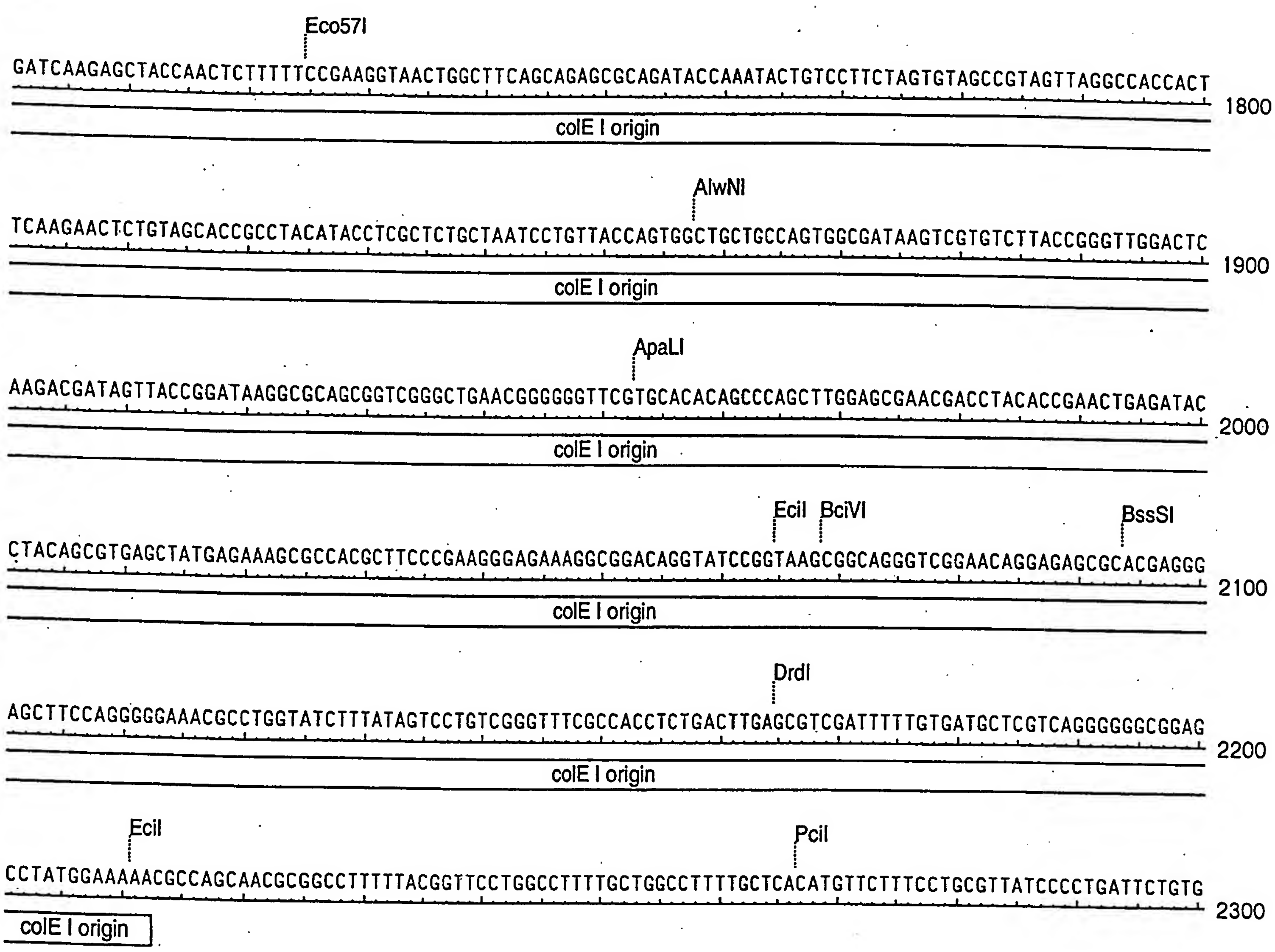
1600
AGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCG
1700
colE I origin

Fig. 21D


Eco57I
AlwNI
ApaLI
EciI
BciVI
BssSI
DrdI
EciI
PciI
colE I origin
colE I origin
colE I origin
colE I origin
colE I origin
colE I origin

GATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACT 1800

TCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTC 1900

AAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATAC 2000

CTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGG 2100

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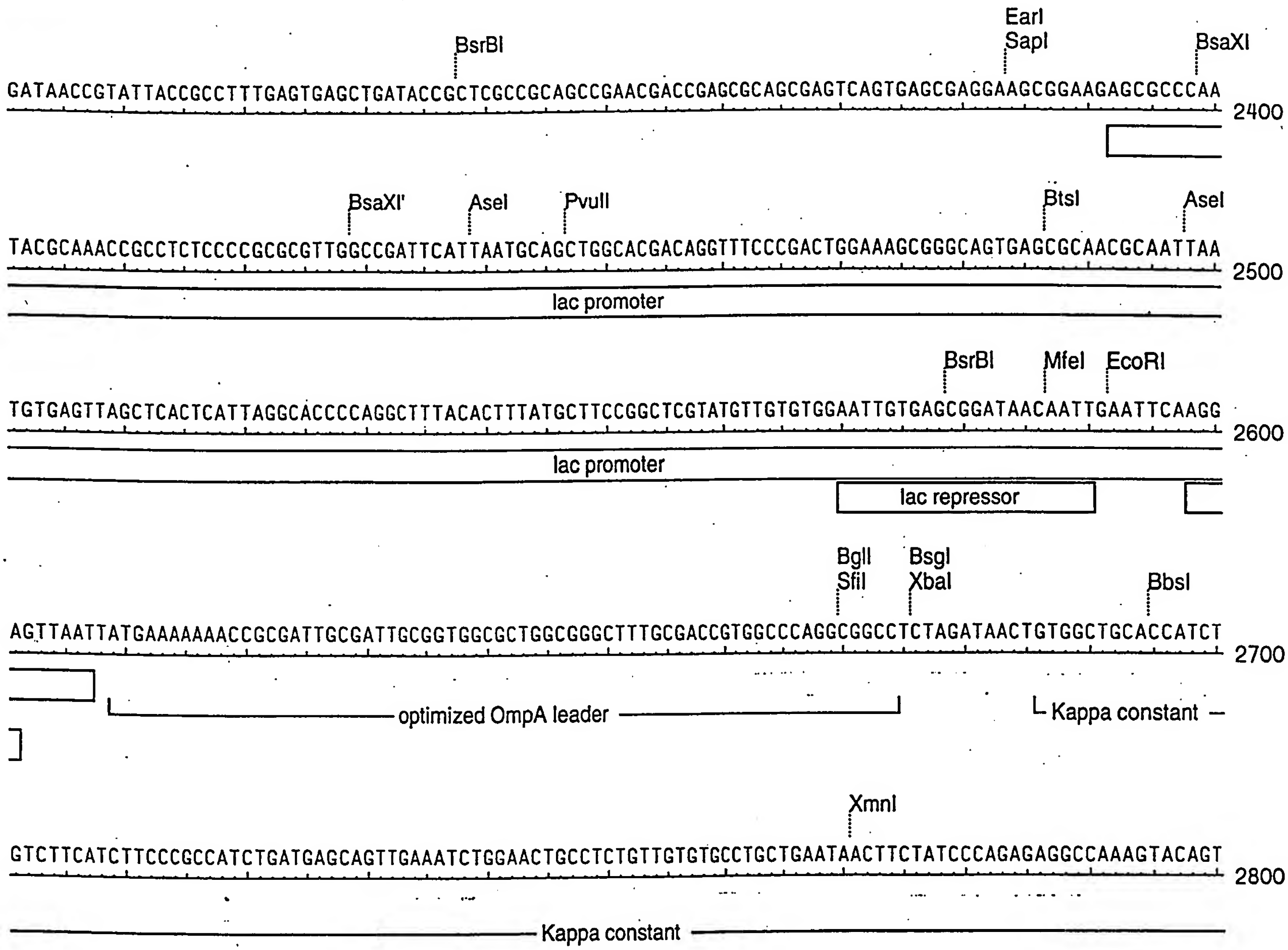
CCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTG 2300

Fig. 21E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EarI
SapI
BsaXI
GATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAA
2400
BsaXI'
AseI
PvuII
BtsI
AseI
TACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAA
2500
lac promoter
BsrBI
MfeI
EcoRI
TGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTGAATTCAAGG
2600
lac promoter
lac repressor
BglI
SfiI
BsgI
XbaI
BbsI
AGTTAATTATGAAAAAAACCGCGATTGCGATTGCGGTGGCGCTGGCGGGCTTTGCGACCGTGGCCCAGGCGGCCTCTAGATAACTGTGGCTGCACCATCT
2700
optimized OmpA leader
Kappa constant
XmnI
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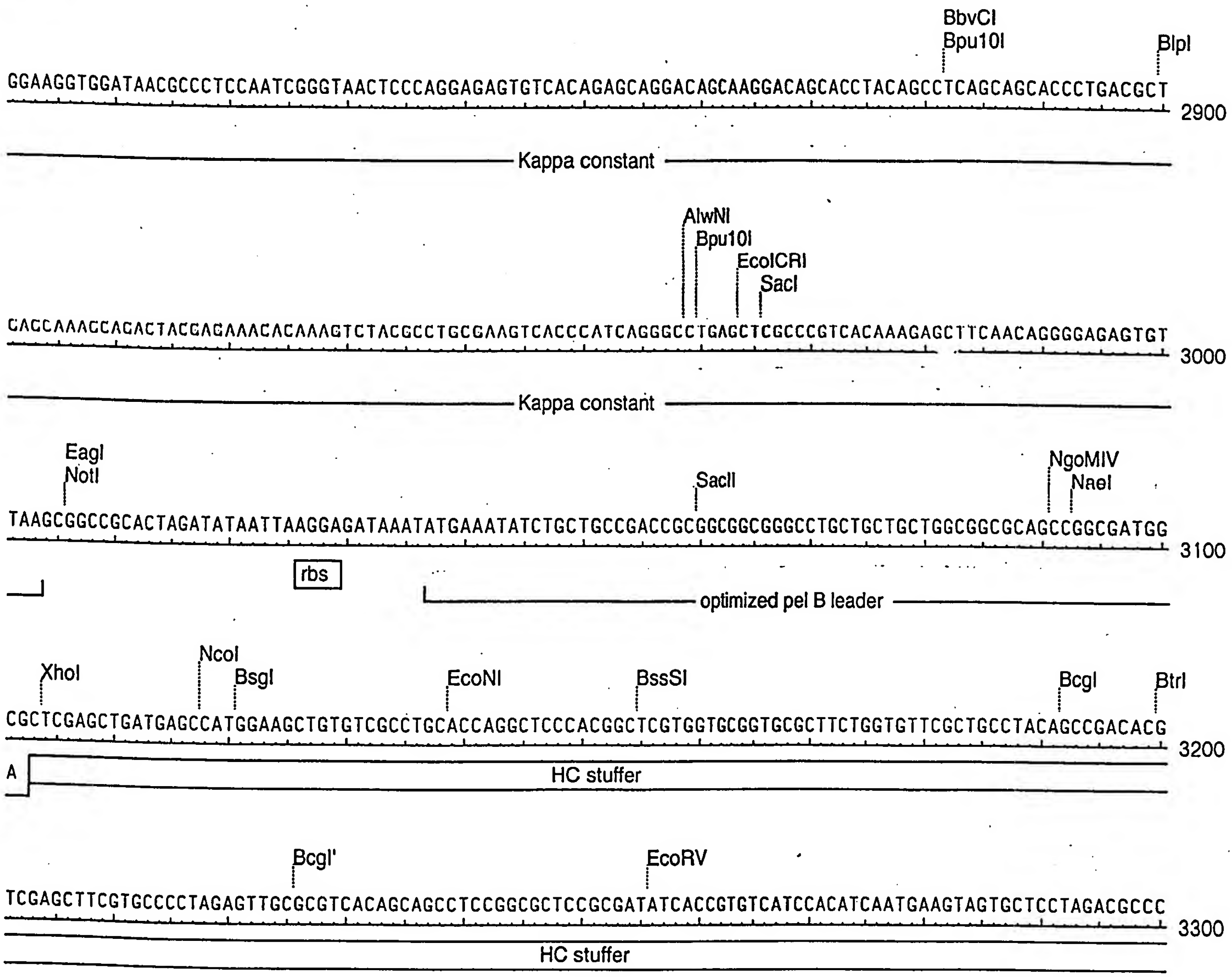
2800
Kappa constant

Fig. 21F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Kappa constant
AlwNI
Bpu10I
EcoICRI
SacI
CACCAAACCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
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Kappa constant
EagI
NotI
SacII
NgoMIV
NaeI
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3100
rbs
optimized pel B leader
XhoI
NcoI
BsgI
EcoNI
BssSI
BcgI
BtrI
CGCTCGAGCTGATGAGCCATGGAAGCTGTGTCGCCTGCACCAGGCTCCCACGGCTCGTGGTGCGGTGCGCTTCTGGTGTTCGCTGCCTACAGCCGACACG
3200
A
HC stuffer
BcgI'
EcoRV
TCGAGCTTCGTGCCCCTAGAGTTGCGCGTCACAGCAGCCTCCGGCGCTCCGCGATATCACCGTGTCATCCACATCAATGAAGTAGTGCTCCTAGACGCCC
3300
HC stuffer

Fig. 21G

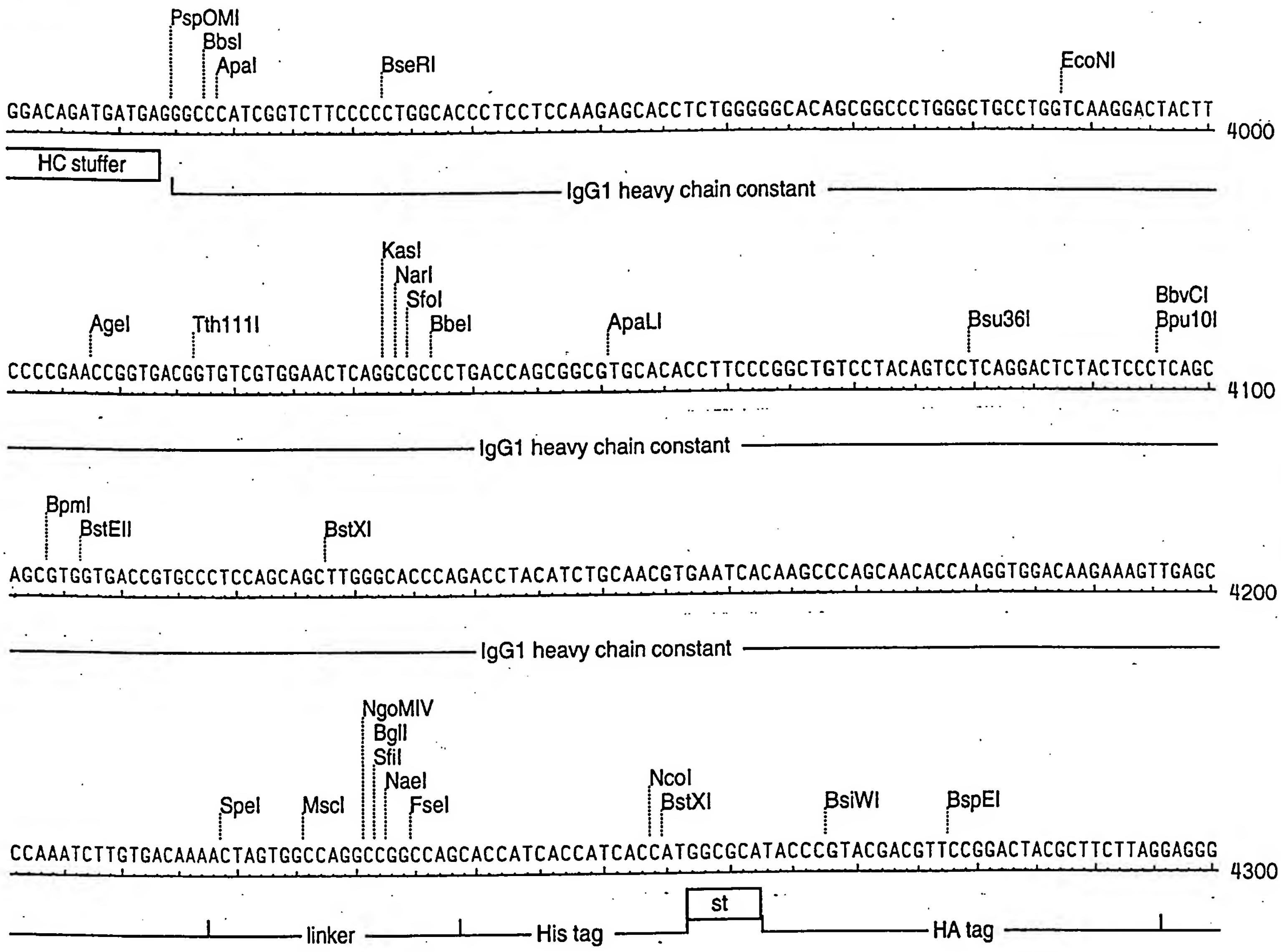
PspOMI
BbsI
ApaI
BseRI
EcoNI
GGACAGATGATGAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTT
4000
HC stuffer
IgG1 heavy chain constant
KasI
NarI
SfoI
BbeI
AgeI
Tth111I
ApaLI
Bsu36I
BbvCI
Bpu10I
CCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGC
4100
IgG1 heavy chain constant
BpmI
BstEII
BstXI
AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGC
4200
IgG1 heavy chain constant
NgoMIV
BglI
SfiI
NaeI
FseI
SpeI
MscI
NcoI
BstXI
BsiWI
BspEI
CCAAATCTTGTGACAAAACTAGTGGCCAGGCCGGCCAGCACCATCACCATCACCATGGCGCATACCCGTACGACGTTCCGGACTACGCTTCTTAGGAGGG
4300
st
linker
His tag
HA tag

Fig. 21H

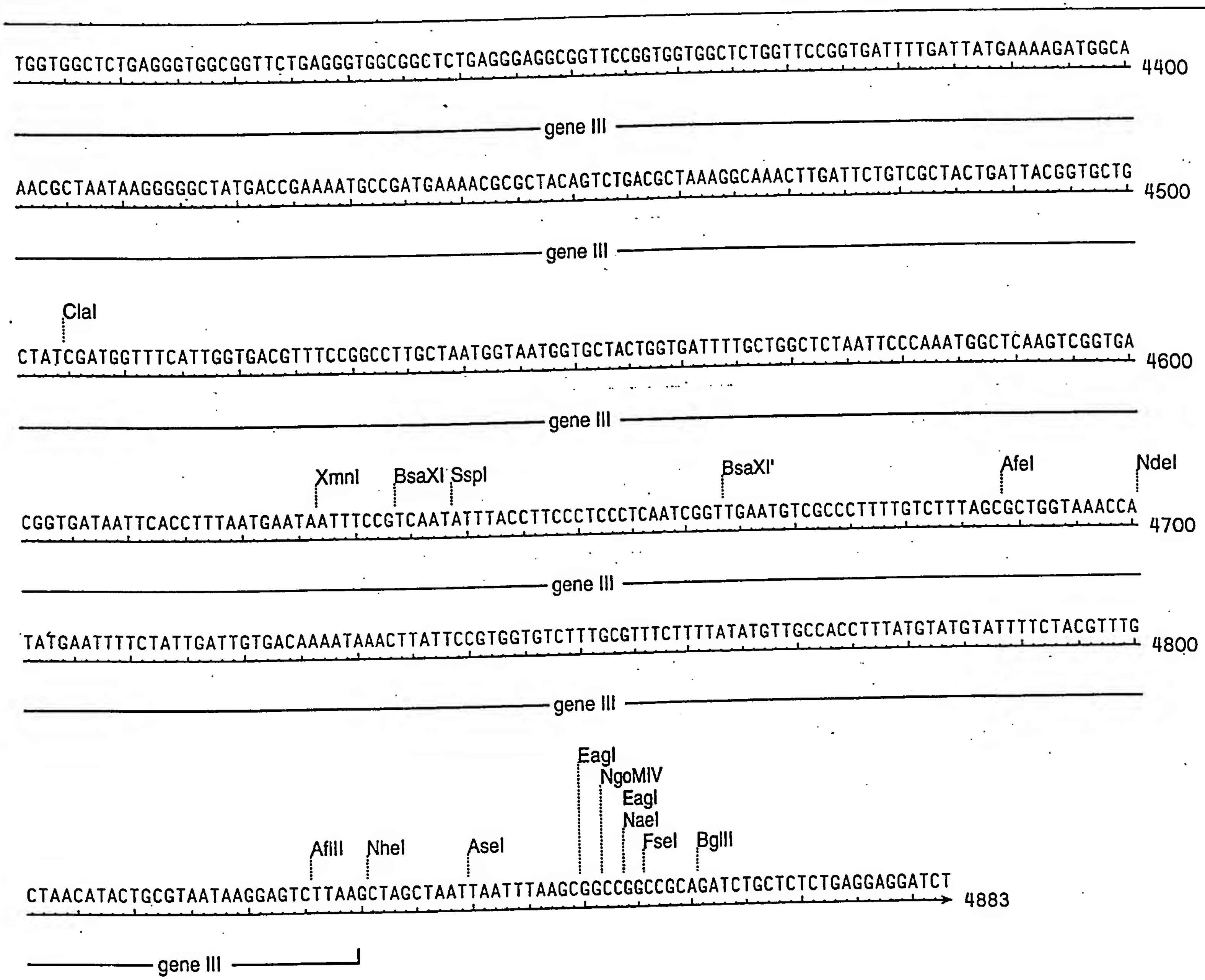
TGGTGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGAGGGAGGCGGTTCCGGTGGTGGCTCTGGTTCCGGTGATTTTGATTATGAAAAGATGGCA
4400
gene III
AACGCTAATAAGGGGGCTATGACCGAAAATGCCGATGAAAACGCGCTACAGTCTGACGCTAAAGGCAAACTTGATTCTGTCGCTACTGATTACGGTGCTG
4500
gene III
ClaI
CTATCGATGGTTTCATTGGTGACGTTTCCGGCCTTGCTAATGGTAATGGTGCTACTGGTGATTTTGCTGGCTCTAATTCCCAAATGGCTCAAGTCGGTGA
4600
gene III
XmnI
BsaXI
SspI
BsaXI'
AfeI
NdeI
CGGTGATAATTCACCTTTAATGAATAATTTCCGTCAATATTTACCTTCCCTCCCTCAATCGGTTGAATGTCGCCCTTTTGTCTTTAGCGCTGGTAAACCA
4700
gene III
TATGAATTTTCTATTGATTGTGACAAAATAAACTTATTCCGTGGTGTCTTTGCGTTTCTTTTATATGTTGCCACCTTTATGTATGTATTTTCTACGTTTG
4800
gene III
AflII
NheI
AseI
EagI
NgoMIV
EagI
NaeI
FseI
BglII
CTAACATACTGCGTAATAAGGAGTCTTAAGCTAGCTAATTAATTTAAGCGGCCGGCCGCAGATCTGCTCTCTGAGGAGGATCT
4883
gene III

Fig. 211